Abstract #362
Poster board #041

Clinical mass spectrometry-based proteomics unlocks personalized medicine potential from bone metastases

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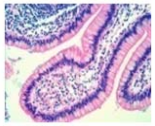
Background

- Bone metastases are rarely used to guide therapy choice; decalcification of bone destroys protein and tissue antigenicity, often precluding traditional molecular analysis.
- Tumors and their bone metastases are biologically distinct; therapies targeted to primary tumors are often ineffective against metastatic lesions.
- We assessed the effects of decalcification on proteomic analysis of tumor tissue using targeted mass spectrometry.
- We also quantified therapeutically-relevant proteins in decalcified bone metastases of cancer patients using mass spectrometry-based proteomics.

Material & Methods



10 µm

Decalcification time (hours)
0, 1, 3, 12, 24

FFPE tumor tissue

Figure 1. Non-bone tissue specimens were processed with and without Decal-Stat™ decalcification solution prior to targeted tumor cell microdissection and mass spectrometric analysis.

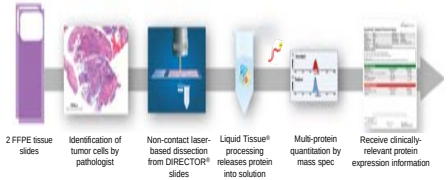


Figure 2. Multiplexed proteomics analysis workflow using Liquid Tissue ™ enabled targeted selected reaction monitoring (SRM) mass spectrometry.

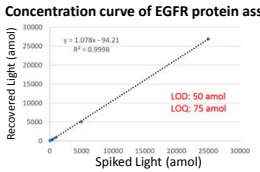
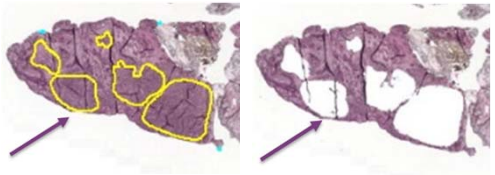


Figure 3. Analytical performance of EGFR assay: the amount of EGFR light peptide recovered (amol) was plotted against the amount of light peptide spiked (amol) to create a concentration curve.

Results

Laser microdissection of bone metastasis biopsies



Tumor marked for microdissection | Tumor sample post microdissection

Figure 4. Microdissection images of FFPE bone biopsy tissue section marked before and after non-contact laser microdissection of targeted tumor cells.

Proteomics unaffected by decalcification conditions

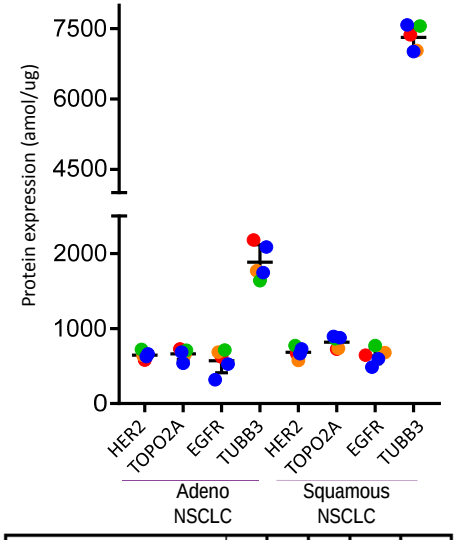


Figure 5. Impact of decalcification on protein quantitation from non-bone tumor tissues (n=2) of 4 protein markers..

Chemotherapy protein markers	Resistance	ERCC1, TUBB3, RRM1, MGMT
	Response	FRa, hENT1, SPARC, TOPO1, TOPO2A
Targeted therapy protein markers	Response: approved therapies	ALK, AR, EGFR, HER2, PDL1, ROS1
	Response: agents in clinical trials	AXL, FGFR2, HER3, IGF-1R, MET, MSLN, RON
Diagnostic protein markers	Lung	CK7, TTF1, CK5, TP63
	HPV infection	P16

Table 1. Multiplexed clinical proteomic menu

Predicted therapeutic benefit of chemotherapies as measured by protein expression in bone metastases of NSCLC patients

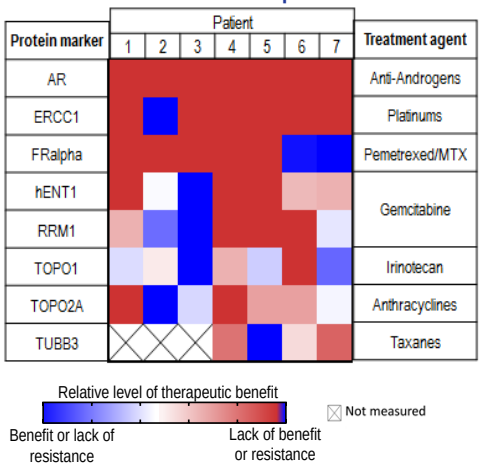


Figure 6. Normalized expression levels of protein markers from decalcified bone metastases of NSCLC patients as measured with SRM mass spectrometry.

Predicted changes in therapeutic response in metastases vs primary

Breast cancer primary with metastasis to the iliac bone			
Protein marker	Quantity (amol/µg)		Treatment implication
	Primary	Mets	
ERCC1	ND	127	May have become platinum resistant
TOPO1	745	1585	May now respond to irinotecan/topotecan
TOPO2A	ND	545	May respond to anthracyclines

Table 2. Difference in protein expression levels between primary tumor and decalcified bone metastasis.

Mandible primary with metastasis to lymph nodes of neck			
Protein marker	Quantity (amol/µg)		Treatment implication
	Primary	Mets	
ERCC1	ND	176	May have become platinum resistant
RRM1	ND	336	
TUBB3	1805	ND	May now respond to taxanes
TOPO2A	ND	1205	May now respond to anthracyclines
TOPO1	ND	1100	May now respond to irinotecan/topotecan
EGFR	980	125	May now be less responsive to anti-EGFR agents

Table 3. Difference in protein expression levels between decalcified primary bone tumor and non-bone (lymph node) metastasis.

Conclusions

- Decalcifying solution had no discernable effects on proteomic quantification of biomarker proteins in archived tumor samples.
- Targeted proteomics quantified an entire panel of therapeutically-relevant proteins from a single decalcified bone biopsy specimen.
- Proteomic analysis of bone metastases upon diagnosis of metastasis or at relapse could inform treatment decisions, particularly in patients with disease progression only in bone lesions or whose bone biomarkers are discordant from those of the primary tumor.

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